31. (Added) A substrate having a surface area, the surface area comprising attached labeled probe molecules, said probe further comprising a fluorescent label.

a4 cancel

32. (Added) The method of claim 16, 17, 18, 20, 22, 23, 24 or 25, wherein the multiple labeled probes and the multiplying of the labeled probes are achieved by a non-amplification step.

REMARKS

I. Preliminary Remarks

Applicant has carefully considered the detailed Office Action and sets forth detailed responses herein. Claim 9 is cancelled. Claims 4-5 and 10-16 have been amended to more particularly point out and distinctly claim certain aspects of Applicant's invention. Applicant notes that the amendments are not intended to change the scope of the claimed invention, rather such amendments are being made partly in response to the rejections under 35 U.S.C. §§ 102,103 and 112. Accordingly, it is respectfully submitted that such amendments do not raise new issues and should be entered in accordance with 37 C.F.R. 1.116(a) and MPEP 714.12 and 714.13.

At this time, Applicant would like to draw attention to the fact that there is no universally acceptable nomenclature with regards to the terms "***targets***" and "***probes***" when used in microarray and DNA chip technology. For example, ***both the present invention and the primary cited reference of record, McGall '501***, utilize the term "***target***" as that which is comprised of nucleic acids, and the term "***probe***" is as that which is also comprised of nucleic acids ***BUT immobilized in arrays on a solid substrate.***

Moreover, to date, what is standard in the art of microarray and DNA chip technologies, is that the term "***target***" is typically a cDNA isolated from cells and tissues. This "***target***" is then hybridized to the "***probe***" fixed in the array. In this general method, ***it is the "target" that is labeled with a fluorescent dye and NOT the probe.***

Still this confusion is further compounded by the fact that in standard molecular biology methods, such as that in Northern and Southern blots, a "target" is fixed onto a substrate and the "probe" is that which is hybridized to the "target". Also in these methods, ***it is the "probe" that is typically labeled NOT the target.***

Thus, although both the present invention and that of conventional molecular biology methods label probes, the term ***probe*** carries a special and different significance.

Applicant submits for reconsideration and allowance of all of the claims in view of the above amendments and the above and following remarks are respectfully requested.

II. Synopsis of the Invention

One main embodiment of the present invention provides ***methods and compositions for labeling probe molecules***. Probe molecules include nucleic acids, amino acids or carbohydrates. More particularly, the label is fluorescent.

In another main embodiment, the present invention provides methods and compositions for ***hybridizing labeled probes*** to target nucleic acids. Also, in the present invention, when the probe is an ***unhybridized labeled molecule***, or single-stranded labeled molecule, it has a ***first level of fluorescence***, wherein the first level of fluorescence is greater than zero.

In still another main embodiment, the present invention provides methods and compositions for ***hybridizing labeled probes*** to target nucleic acids. When the labeled probe is hybridized, or becomes double stranded, to its complementary target molecule the fluorescence is has a second level of fluorescence, wherein the second level of fluorescence approaches zero.

Note that methods and compositions of the present invention for ***labeling, detecting and quantifying probes***, is ***opposite*** to the standard methods and compositions in the art of microarray and DNA chip technologies, which ***label targets***.

III. Rejection Under 35 U.S.C. § 112 Second Paragraph

Claims 14 and 15 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite. Claims 14 and 15 have been amended in accordance with the Examiner's remarks and to improve their form. The amendments clarify that claims 14 and 15 now contain sufficient antecedent basis by being depended from claim 13. Applicant respectfully submits that claims 14 and 15 are now in good order for allowance.

IV. The Rejections Under 35 U.S.C. § 102(e) and 103(a)

A. Rejection over McGall '501 under 35 U.S.C. § 102(e)

Claims 1, 2, 4, 5, 8, 9 and 13 stand rejected under 35 U.S.C. §102(e) as being anticipated by U.S. Patent # 6,156,501to McGall et al., (McGall '501). As previously mentioned, claims 4-5 and 13 have been amended. Claim 9 has been cancelled. Applicant respectfully submits that these claims are now in good order for allowance.

Briefly, McGall '501 provides "probes comprised of nucleotide analogues immobilized in arrays attached to solid substrates...and target nucleic acids comprised of nucleotide analogues."

It is stated in the Office Action that because McGall '501 teaches "probes comprised of nucleotide analogues immobilized in arrays on solid substrates... and target nucleic acids comprised of nucleotide analogues... ", McGall's invention reads on the present invention. ***However***, McGall '501 teaches that "hybridization is detected ***by labeling a target*** with e.g., fluorescein or other known visualization agents and incubating the target with an array of oligonucleotide probes (column 12, lines 39-42). This is in ***complete contrast*** to the present invention, which provides methods for labeling ***probes*** NOT ***targets*** (independent claim 1).

Stated very simply, a key embodiment of the present invention is ***labeling of probes***, arrayed on a solid substrate, and ***NOT targets***. Labeling targets is standard in the art of microarray and DNA chip technology and McGall '501. Hence, the present invention by labeling probes, is not anticipated by McGall '501, which labels targets.

1) Claim 1

For example, independent claim 1 comprises a substrate having a surface area, the surface area comprising attached labeled probe molecules. ***McGall '501 does not teach the labeling of probes, McGall '501 teaches the labeling of targets*** (column 12, line 39-40)

Since McGall '501 does not anticipate independent claim 1, it follows that dependent claims 2, 4, 5 and 8, being dependent on independent claim 1, are also not anticipated by McGall '501.

2) Claim 2

For instance, dependent claim 2 states that the labeled probe molecules are fluorescent. ***McGall '501 does not teach labeling probes***, therefore, they do not teach fluorescent labeling of probes.

3) Claim 4

Similarly, dependent claim 4 describes fluorescently labeled probes are comprised of native and nonnative nucleotides. ***McGall '501 does not teach labeling probes***, therefore, they do not teach that probes can be either native or nonnative nucleotides. In the present invention, the fluorescent label is optionally incorporated by oligonucleotide synthesis or enzymatic reactions (page 4, lines 10-11).

Another significant difference between the present invention and that of McGall '501, is that McGall '501 states that "nucleotide analogues are preferably incorporated into target nucleic acids using in vitro amplification methods such as PCR, LCR... and the like" (column 10, line 52-56). ***McGall '501, teaches that labeled targets are comprised of genomic DNA, cDNA, unspliced RNA, mRNA and rRNA*** (claim 41). ***McGall '501 also teaches that the target nucleotide is amplified prior to said hybridization step*** (claim 42).

This in ***direct contrast to the present invention***, which provides methods for labeling probes, which are comprised of nucleic acids and nucleic analogues that are ***not derived from genomic DNA, cDNA, unspliced RNA, mRNA or rRNA, and do not need to be amplified prior to use in hybridizations with targets.***

Typically in standard microarray and DNA chip technologies and in McGall '501, the amount of target available is solely dependent on the amount of starting genomic material including DNA, cDNA, RNA, etc., available (claim 41). These methods also amplify the starting nucleic acid material by using PCR and LCR to increase the number of target molecules. Similarly, McGall '501 has to amplify the number of targets because there is not sufficient number of target molecules initially isolated from cells and tissues (claim 42). Increase in the number of target molecules translates to increase in the number of labeled target molecules and a subsequent increase in the level of detection.

In contrast, the present invention, by incorporating label into oligonucleotides via automated synthesis, avoids the problem of not having enough starting material all together. Therefore, the present invention is an improved method over that of the present invention because it eliminates the typical step of labeling targets and amplifying targets.

5) Claim 5

Dependent claim 5 states that fluorescent nucleotide analogues used to label probes includes more than one fluorescent molecule including 2-amino purine (dependent claim 6). McGall '501 by providing methods for labeling targets and does not suggest labeling probes, thus using fluorescent molecules to label probes comes from the present invention, not McGall '501

6) Claim 8

Still, in another example, dependent claim 8 comprises a probe that is a carbohydrate. It is the Examiner's position that because nucleotides are carbohydrates, claim 5 is obvious over McGall '501. Applicant submits that nucleic acids and carbohydrates are very different organic molecules. For instance, carbohydrates have a general formula of Cn(H20)n. Whereas, nucleic acids are nitrogen-containing bases attached to sugar molecules and a phosphate group. Only the sugar residue is considered a carbohydrate, as carbohydrates do not contain nitrogen or phosphorus. Thus, McGall '501 does not teach the present invention of providing for carbohydrate labeled probes, and the present invention is thereby not obvious over the cited reference.

7) Claim 9

Claim 9 has been cancelled.

8) Claim 13

Lastly, amended claim 13 teaches approximate size of bead substrates being about 10 microns to 20 microns. McGall '501 fails to teach the sizes of bead substrates and McGall '501 does not anticipate the present invention.

Therefore, amended claims 4-5, 8 and 13 are not anticipated by, or alternatively, not obvious, over McGall '501. Applicant respectfully submits that the rejection of these claims under 35 U.S.C. § 102(e) be withdrawn.

B. Rejection over McGall '501 under 35 U.S.C. § 103(a)

Claims 3, 10-12 and 16 stand rejected under 35 U.S.C. §103(a) as being obvious over McGall '501. Claims 10-12 and 16 have been amended, all claims are now in good order for allowance.

Dependent claim 10 has been amended so that it is depended on independent claim 1 and not on claim 9, which has been cancelled. Dependent claims 11-12 have been amended to depend on claim 10 and not on claim 9, which has been cancelled.

1) Claim 3

Applicant submits that rejection to dependent claim 3 has been obviated as discussed previously in the section for rejection of independent claim 1 under 35 U.S.C. § 102(e) with reference to the ***present invention providing methods to label probes NOT targets***. Thus, McGall '501 by not teaching the labeling of probes, does not teach the labeling of probes with fluorescent labels in the range of about 300 nm to about 700 nm.

2) Claim 10

In claim 10, it is the Examiner's position that separating the microarrays into quadrants was well known at the time of the invention. However, McGall '501 states that probes are "usually laid down in ***rows and columns*** for simplified data processing (column 15, lines 48-49)." Dividing arrays into ***quadrants*** comes from the present invention not from McGall '501. Any association of dividing arrays in quadrants is taught in the present disclosure and not from the cited reference. Hence, the use of hindsight is prohibitive for rejection under 35 U.S.C. § 103(a).

3) Claim 11 & 12

Dependent claim 11 teaches probes being located on an array having about 100 to about 10,000 different quadrants. Further, dependent claim 12 teaches methods of making an array of about 100 to about 1000 and labeled probes. It is the Examiner's position that McGall '501 teaches arrays with ***at least 1000*** nucleotide analogue probes (claims 13 and 72). Although, there is an overlap of the number of probes on any array, the general scope of the present invention and that of McGall '501 is significantly different such that this overlap is not obvious over McGall '501; because ***McGall '501 teaches methods for labeling targets*** . Also, as mentioned above, the teachings of quadrants on arrays comes from the present invention and not McGall '501.

4) Claim 16

Lastly, amended independent claim 16 recites methods for accessing the presence of a target molecule in a cell or tissue comprising a plurality of steps including procuring an array with attached labeled probes. McGall '501 does not teach ***labeling of probes***, McGall '501 teaches ***labeling of targets***. Also, in the present invention, the first detection step with the labeled probe alone is more fluorescent than the target-probe hybrid that forms in subsequent steps. The target-probe hybrid actually has no detectable fluorescence or is very close to zero (Independent claim 16, step g).

Thus, the present invention by providing methods of labeling probes and a different and improved method of detecting target-probe hybrids is not obvious over McGall '501, which provides for labeling of targets and a standard method of detecting target-probe hybrids.

Therefore, claim 3 and amended claims10-12 and 16 are not obvious over McGall '501 because of the above responses. Applicant respectfully submits that the rejection of these claims under 35 U.S.C. § 103(a) be withdrawn.

C. Rejection over McGall in view of Gelfand '375

Claim 6 stands rejected under 35 U.S.C. §103(a) as being obvious over McGall '501 and further in view of U.S. Patent No. 5, 804, 375 to Gelfand et al., (Gelfand '375). Applicant respectfully submits that after careful analysis of McGall '501 and Gelfand '375, there is no suggestion, motivation and/or incentive to combine the cited references to arrive at the present invention as required under 35 U.S.C. §103(a).

Dependent claim 6 is indirectly dependent on independent claim 1, which provides for labeled probe molecules. Dependent claim 6 further states that the labeling molecule is 2-amino purine.

First, ***McGall '501 teaches methods of labeling targets and NOT probes.*** Whereas, although Gelfand '375 labels probes (column 12, lines 35-40), ***it is the standard molecular biology use of the word "probe"***, similar to that used in Northern and Southern blots (terminology of probe and target discussed above), and not the same meaning of the word probe as used in the present invention and McGall '501. Furthermore, Gelfand '375 requires an amplification step (column 12, lines 35-40). In the present invention, incorporation of 2-amino purine into probes ***does not require an amplification or PCR step.***

Therefore, claim 6 is not obvious over McGall '501 or Gelfand '375 alone or in combination, because of the above responses. Applicant respectfully submits that the rejection of this claim under 35 U.S.C. § 103(a) be withdrawn.

D. Rejections over McGall in view Scholin '530

Claim 7 stands rejected under 35 U.S.C. §103(a) as being obvious over McGall '501 and further in view of U.S. Patent No. 6, 187, 530 B1 to Scholin (Scholin '530). Applicant respectfully submits that after careful analysis of McGall '501 and Scholin '530, there is no suggestion, motivation and/or incentive to combine the cited references to arrive at the present invention as required under 35 U.S.C. §103(a).

Claim 7 states that probes are comprised of amino acids. Again, as discussed above, the labeling of probes in the present invention is different from the labeling of probes in Scholin '530. Also, although antibodies are amino acids and antibodies in Scholin '530 function as probes, it is not analogous to the present invention and its use of probes. In the present invention probes are comprised of nucleic acids (claim 4), nucleic acid analogues (claim 5), amino acids (claim 7) and carbohydrates (claim 8). Whereas, in Scholin '530 if the antibody was anything other than a plurality of amino acids, it would no longer be an antibody, an therefore, no longer a probe. Thus, the present invention by providing different compositions for probes is different from and not analogous to Scholin '530 providing for only antibody probes.

Moreover, one cannot use the instructions of the present invention to piece together the teachings of the prior art. More particularly, one cannot use hindsight reconstruction to pick and choose among isolated disclosures to depreciate the claimed invention. *In re Fine*, 837 F.2d 1071, 1075, 5 U.S.P.Q.2d (BNA) 1596, 1600 (Fed. Cir. 1988).

E. Rejection over McGall '501 in view of Mandecki '571

Claims 14 and 15 stand rejected under 35 U.S.C. §103(a) as being obvious over McGall '501, and further in view of U.S. Patent No. 6,001,571 to Mandecki (Mandecki '571). As previously mentioned, claims 14 and 15 have been amended and are now in good standing for allowance.

Again, Applicant respectfully submits that after careful analysis of McGall '501 and Mandecki '571, there is no suggestion, motivation and/or incentive to combine the cited references as required under 35 U.S.C. §103(a). For example, ***McGall '501 does not teach or disclose the size of the bead as in the present in invention*** (dependent claim 13). ***Mandecki***

'571 does not teach or disclose the size of the beads nor do they provide guidance that the beads in their invention are comprised of ferromagnetic metal cores; other than sole mention of the word "ferromagnetic" when discussing the prior art in the background (column 1, page 21-26). Thus, since McGall '501 and Mandecki '571 ***do not teach any element*** of dependent claims 14 and 15, alone or in combination, there is no 35 U.S.C. §103(a) rejection of obviousness.

Also, as with the previous argument rejecting claim 7, one cannot use hindsight reconstruction to pick and choose among isolated disclosures to depreciate the claimed invention. *In re Fine*, 837 F.2d 1071, 1075, 5 U.S.P.Q.2d (BNA) 1596, 1600 (Fed. Cir. 1988).

V. In Summary

In summary, claims 2, 4-5 and 10-16 have been amended. Claim 9 has been cancelled. Claims 20 –32 have been added.

The present invention teaches methods and compositions including: a substrate having a surface area comprising attached probes; said probes are labeled with fluorescence molecules and multiplied using non-amplification method; detecting a first level of fluorescence of labeled probe that is greater than zero; hybridizing fluorescently labeled probes to targets; and detecting second level of fluorescence of labeled probes that is approximately zero or lower than the first level fluorescence or close to background levels. Therefore, the present invention ***is patentable and not obvious over the cited references of record***. The present invention is patentable and not obvious over McGall '501 because teaches ***labeling targets, amplifying targets using PCR or LCR*** , hybridizing labeled targets to probes in arrays and detecting fluorescence levels after hybridization. The present invention is patentable and not obvious over Gelfand '375 because although Gelfand 375 teaches labeling nucleic acid probes, ***the word probe is used according to the standard molecular biology meaning of the word*** (as previously discussed), and not the same meaning as the present invention and that of McGall '501. The present invention is patentable and not obvious over Scholin '530 or Mandeck '571 because Scholin '530 teaches antibody probes which can ***only*** be comprised of amino acids, whereas the present invention probes can include nucleotides, carbohydrates and amino acids; and Mandecki '571 teaches surface area in the form of beads, but ***does not teach beach size,*** whereas the present invention teaches that bead sizes have a range of about 10 microns to about 20 microns.

The present invention, therefore, is patentable and not obvious over the prior art of record, alone or in combination, because the prior art of record does not teach the elements of the present invention (discussed above).

Also, claims 20-32 have been added to better define the present invention without changing the overall scope.

Applicant submits that the Examiner has not addressed claims 17-19. The claims have neither been allowed nor rejected by the Examiner.

VI. Conclusion

In view of the above, it is submitted that this application is now in good order for allowance, and such early action is respectfully solicited. Should matters remain which the Examiner believes could be resolved in a telephone interview, the Examiner is requested to telephone the Applicant's undersigned attorney.

Respectfully submitted,

Date: July 18, 2001

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ADDENDUM PAGES

SET OF SUBMITTED CLAIMS WITH UNDERLINING AND/OR BRACKETS

1. A substrate having a surface area, the surface area comprising attached labeled probe molecules.

2. (Amended) The substrate of claim 1 wherein the labeled probe [molecules of claim 1] is fluorescent.

3. The substrate of claim 1 wherein the labeled probe [molecules of claim 1 wherein label] fluoresces at a wavelength of about 300 nm to about 700 nm.

4. (Amended) The substrate of claim 1 wherein the labeled probe [molecules are] is comprised of native and nonnative nucleotides.

5. (Amended) The labeled probe molecules of claim 1 wherein the nucleotides are nucleotide analogs including 2 –amino purine at least for adenosine or guanine; ribonucleoside or 2,6-diamino ribonucleoside, formycin A, formycin B, oxyformycin B, toyocamycin, sangivamycin, pseudoouridine, showdomycin, minimycin, pyrazomycin, 5-amino-formycin A, 5-amino-formycin B or 5-oxo-formycin A at least for adenosine; 4-amino-pyrazolo [3, 4d] pyrimidine, 4,6-diamino-pyrazolo [3, 4d] pyrimidine, 4-amino-6-oxo-pyrazolo [3,, 4d] pyrimidine, 4-oxo-pyrazolo [3, 4d] pyrimidine, 4-oxo-6-amino-pyrazolo [3, 4d] pyrimidine, 4,6-dioxo-pyrazolo [3, 4d] pyrimidine, pyrazolo [3, 4d] pyrimidine, 6-amino-pyrazolo [3, 4d] pyrimidine or 6-oxo-pyrazolo [3, 4d] pyrimidine at least for cytosine or thymidine

6. The labeled probe molecules of claim 2, wherein the nucleotide analog is 2-amino purine.

7. The substrate of claim 1 wherein the labeled probe molecules are comprised of amino acids.

8. The substrate of claim 1 wherein the labeled probe molecules are comprised of carbohydrates.

9. (Cancel) The substrate of claim 1 wherein the substrate is a microarray.

10. (Amended) The substrate of claim 1 wherein the substrate is a microarray [of claim 9] further having the surface area divided into quadrants wherein each different quadrant has different labeled probe molecules [of different sequences].

11. (Amended) The microarray [9] substrate of claim 10 having from about 100 to about 10,000 different labeled probe [molecule] molecules [sequences] located upon about 100 to about 10,000 different quadrants.

12. (Amended) The microarray of claim [9]10 having about 100 to about 1,000 labeled probe [molecule] molecules per quadrant.

13. (Amended) The [microarray] substrate of claim 1 wherein the substrate is a bead, said bead sizes range from about 10 microns to about 20 microns.

14. (Amended) The bead substrate of claim [6] 13 wherein the bead is formed of a ferromagnetic metal core and a polymeric coating.

15. (Amended) The bead substrate of claim [7] 13 having from about 100 to about 1,000 labeled probe [molecule] molecules attached to the surface area of the bead.

16. (Amended) A method for assessing the presence of a target molecule in a cell or tissue sample comprising the steps of:

a. procuring a microarray having a surface area comprising attached labeled probe molecules in quadrants;

b. detecting the level of label expressed within each quadrant a first time;

c. applying a sample comprising unlabeled target sequences to the microarray;

d. providing sufficient conditions and time for target molecules to selectively pair with the complementary labeled probe molecules; and

e. detecting the level of label expressed within each quadrant a second time;

f. comparing the levels of label expressed between the first time and the second time for each quadrant.

g. repeating steps c - f until the levels of label approaches zero and/or about background levels;

h. the difference between levels of label in that of step f and that of step c identifies a target/probe pair.

17. A method quantifying the amount of a target molecule in a sample comprising the steps of:

a. procuring a first substrate having a surface area comprising a known number of labeled probe molecules;

b. detecting the level of label expressed by the labeled probe molecules on the substrate;

c. contacting a substrate with a volume of sample containing unlabeled target nucleotide sequences;

d. providing sufficient conditions and time for target molecules to selectively pair with the labeled probe molecules;

e. removing the substrate from the sample and detecting the level of label expressed by the substrate after exposure to the sample;

f. where the level of label expression of the first substrate is substantially reduced to levels substantially similar to background levels, repeating steps a. through e. with subsequent substrates, having surface areas comprising a known numbers of labeled probe molecules.

g. Calculating the amount of target molecule in the volume of sample by adding the known number of labeled probe molecules present on the first substrate and subsequent substrates contacted with the sample, wherein the levels of label expression of the substrates were reduced relative to the levels prior to contacting the sample.

18. The method of claim 10, wherein the level of label expression is evaluated using a flow cytometer.

19. A substrate having a surface area divided into quadrants;

different nucleotide probe molecule sequences bound to the surface area, wherein different nucleotide probe molecule sequences are bound to distinct quadrants;

wherein the nucleotide probe molecules are characterized as being a single stranded form or double stranded in form, wherein the level of label expressed from the single stranded probe molecules is greater than the level of label expressed from the double stranded probe molecules; and

wherein the nucleotide probe molecules are further characterized by an ability to hybridize to target nucleotide sequences.

20. (Added) A method for monitoring the hybridization of target and probe by complementation, said method comprising of:

a. incorporating fluorescent molecules into probes;

b. detecting a first level of label in probe of step a;

d. hybridizing a target with said labeled probe;

e. detecting a second level of label after hybridization of probe and target;

f. comparing the first and second levels of label between that of step b and that of step e, and wherein said difference between second and first levels is less than said first level of step b;

g. washing of unhybridized target;

h. repeating steps d - g until the difference between the first and second levels of label approaches approximately zero and/or about background levels.

21. (Added) A microarray substrate wherein the substrate is a bead, said bead having a surface area comprising attached probe molecules with a fluorescence label, said bead sizes range from about 10 microns to about 20 microns.

22. (Added) A method for monitoring the hybridization of a probe and a target, providing a probe with a fluorescence label and providing a detectable first level of fluorescence of the labeled probe, and providing a second level fluorescence of the labeled probe when hybridized to a complementary target, wherein the second level is lower than the first level.

23. (Added) A method for monitoring the hybridization of a probe and a target, providing a probe with a fluorescence label and providing a detectable first level of fluorescence of the labeled probe, and providing a second level fluorescence of the labeled probe when hybridized to a complementary target, wherein the second level is significantly lower than the first level.

24. (Added) A method for monitoring the hybridization of a probe and a target, providing a probe with a fluorescence label and providing a detectable first level of fluorescence of the labeled probe, and providing a second level fluorescence of the labeled probe when hybridized to a complementary target, wherein the second level is approximately zero.

25. (Added) A method for monitoring the hybridization of a probe and a target, providing a probe with a fluorescence label and providing a detectable first level of fluorescence of the labeled probe, and providing a second level fluorescence of the labeled probe when hybridized to a complementary target, wherein the second level is approximately zero and the first level is greater than zero.

26. (Added) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence.

27. (Added) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target, wherein the second level is lower than the first level.

28. (Added) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target, wherein the second level is significantly lower than the first level.

29. (Added) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target, wherein the second level approaches zero.

30. (Added) A substrate having a plurality of probes, wherein said probes are fluorescently labeled, the labeled probe providing a detectable first level of fluorescence, and when hybridized to a complementary target level, wherein the second level approaches zero and the second level is greater than zero.

31. (Added) A substrate having a surface area, the surface area comprising attached labeled probe molecules, said probe further comprising a fluorescent label.

32. (Added) The method of claim 16, 17, 18, 20, 22, 23, 24 or 25, wherein the multiple labeled probes and the multiplying of the labeled probes are achieved by a non-amplification step.